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Molecular Dynamics of A Biglycan-Rosmarinic Acid Complex with Focal Adhesion Kinase for Possible Arrest of Metastasis in Non-Small Cell Lung Cancer (NSCLC): An *In- Silico* Study

Highland Hyacinth*[1], Thakur Monica [2], Pandya Pujan [3], Mankad Archana [3], George Linz-Buoy [1]

[1] Department of Zoology, Biomedical Technology and Human Genetics, School of Sciences, Gujarat University, Ahmedabad-380009, Gujarat, India

[2] Department of Biochemistry, School of Sciences, Gujarat University, Ahmedabad-380009, Gujarat, India

[3] Department of Botany, Bioinformatics, School of Sciences, Gujarat University, Ahmedabad-380009, Gujarat, India

ABSTRACT

Background: Non-small cell lung cancer (NSCLC) is the major cause of mortality all over the world. Significant increase of biglycan is seen in the lung cancer cells when compared with the normal cells. It promotes tumor invasion and metastasis by activating Focal Adhesion Kinase (FAK) signaling pathway. The increased FAK activity may contribute to the metastatic potential of malignant tumors. This study was carried out to establish binding interactions of some selected phytocomponents against biglycan for the possible arrest of metastasis.

Methods: Protein-ligand interaction studies were performed using 30 natural compounds from different culinary herbs having potential therapeutic role against the target protein biglycan (BGN).

Molegro Virtual Docker (v 5.0) was used as docking tool to evaluate the effectiveness of selected phytocomponents based upon the interaction with the protein's active site residues with minimal binding energy. Protein-protein docking was performed to observe the interaction of BGN and FAK using Hex (v 8.0.0). Molecular dynamics (10 ns) of BGN-RA-FAK and FAK-RA-BGN was performed in Yasara structure (v 17.8.15) which showed stability of the structure in terms of RMSD values.

Results: Molecular docking analysis revealed the selectivity of Rosmarinic acid (RA) towards BGN and FAK. Molecular dynamics trajectory of BGN-RA-FAK and FAK-RA-BGN complexes showed the stability of structure in terms of Time vs Energy and Time vs RMSD values and revealed that binding of RA to BGN will block the interaction of FAK.

Conclusions: Hence, investigating the binding interactions of BGN-RA-FAK complex may turn out to be helpful in arresting metastasis in NSCLC.

Keywords: *Non-small cell lung cancer, Biglycan, Focal adhesion kinase, Phytocomponents, Molecular Docking, Molecular Dynamics*

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***Address for Correspondence:**

Highland Hyacinth, Department of Zoology, Biomedical Technology and Human Genetics, School of Sciences, Gujarat University, Ahmedabad-380009, Gujarat, India

INTRODUCTION

Lung cancer is the leading cause of cancer related deaths and non-small cell lung cancer (NSCLC) is the most frequent subtype comprising of 85% of recorded cases [1]. According to the cancer facts and figures [2] of American cancer society, the five year survival rate for lung cancer is only 18%. It constitutes about 6.9 per cent of all new cancer cases and 9.3 percent of all cancer related deaths in both sexes in India [3] and continues to be a major global health problem. The disease is diagnosed in more than 1.6 million new patients each year. However, significant progress is ongoing in both the prevention and treatment of lung cancer [4]. Many integrated multidisciplinary approaches to the treatment of lung cancer have improved patient outcomes, but there is an urgent need for a novel therapeutic strategy for lung cancer as there is no breakthrough treatment with long-term efficacy [5]. Specifically improved proteomics techniques for the identification, detection, and verification of biomarkers

have improved our understanding of lung cancer but, early detection of lung cancer is important for a better outcome and to decrease the lung cancer mortality rate [6, 7].

Metastasis, the spread of cancer through lymph and blood, to distant organs is the main reason of death for most of the patients with malignancy [8]. Hence, this study was aimed at employing an *in silico* approach to identify a specific binding interaction of Protein-Ligand-Protein for arresting metastasis in NSCLC.

Biglycan (BGN) is a member of small leucine-rich proteoglycans family, and it has been ascribed a pivotal role in oncogenesis and development of various other types of human cancer. It is also an important component of the extracellular matrix [9]. BGN is found in almost every organ in a non-uniform distribution pattern within human body. Recent studies have indicated that there is significant increase of BGN in tumor tissues as compared to normal tissues, in several cancer types. It is known as a multivalent proteoglycan which promotes tumor invasion and metastasis by activating the Focal Adhesion Kinase (FAK) signaling pathway. The increased FAK activity may contribute to the metastatic potential of malignant tumors [10]. Hence, this makes biglycan a potential target protein for the present *in silico* study aimed at halting metastasis.

In this study, molecular docking, Molecular dynamics and protein-protein interaction studies were employed to establish binding interactions of some selected phytocomponents against the target protein biglycan.

MATERIALS AND METHODS

In the present study, Molegro Virtual Docker (MVD) v 5.0 (www.molegro.com) was used as a docking tool, along with this Molegro Molecular Viewer (MMV) was used for calculating docking score [11]. MVD software requires the receptor and ligand coordinates which is either in Mol2 or PDB format. Protein- Protein docking was done with the help of Hex (version 8.0.0) software. Protein- ligand docking and Protein- Protein docking is a very useful approach to find the binding interaction of target protein with single or multiple ligands. For molecular dynamics study YASARA Structure (version 17.8.15) was the software of choice [12]. AMBER14 force field[13] was used for Molecular dynamic simulations [14].

Protein and Ligand Preparation:

The X-ray crystal structures of BGN (PDB ID: 2FT3) [15] and FAK (PDB ID: 4NY0) [16] were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) (http://www.pdb.org/) protein data bank (PDB) [17] having Uniprot ID P21810 and Q05397 respectively (Figure 1) and prepared for molecular docking simulation by removing all the heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.). The crystallographic structures of these proteins were selected according to the maximum matched sequences among the protein chains available in RCSB PDB database. 3D structures of all the 30 natural compounds from different culinary herbs having potential therapeutic role were used as ligands for this study (Figure 2) were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [18] in 3D structure data format (SDF).

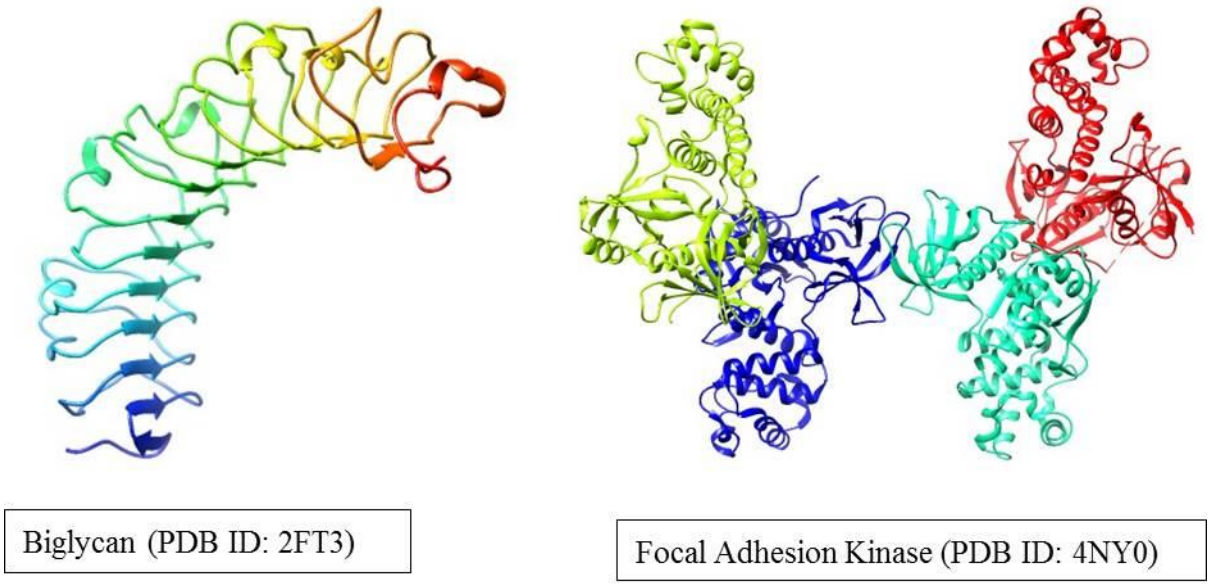


Figure 1: 3D structures of Biglycan (BGN) and Focal adhesion kinase (FAK)

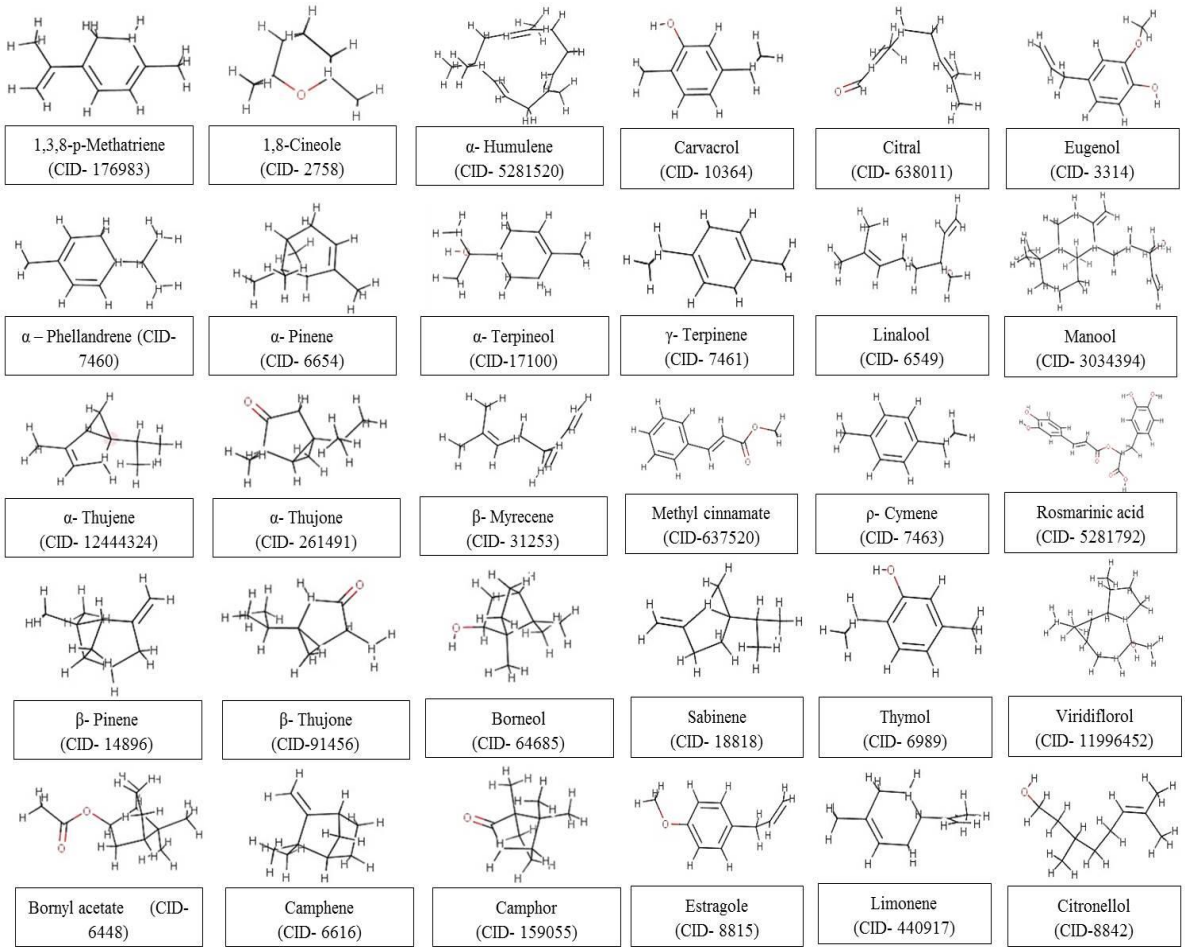


Figure 2: 3D structures of the selected phytocomponents from different culinary herbs

Molecular Docking studies:

(A) Biglycan (BGN) and Phytocomponents (Ligands):

Molecular docking technique was employed to dock the selected phytocomponents against BGN using Molegro Virtual Docker (MVD) to locate the interaction between the phytocomponents and the target protein BGN. All the 30 phytocomponents were docked against the target protein BGN and the compounds having minimum energy were selected for further study. Default settings were used for all the calculations.

(B) FAK and selected phytocomponents (Ligands):

The selected 30 phytocomponents from different culinary herbs were again docked against the targeted protein FAK to check the binding interaction of the phytocomponents with the protein.

(C) BGN and FAK (Protein- Protein) Docking:

Protein- Protein docking of BGN and FAK was done using Hex (version 8.0.0) protein docking software. 500 model structures were formed according to the interaction energy and bonding of the two proteins. The highest docking score was used for the further study.

(D) Molecular docking of BGN-Phytocomponent complex with FAK and FAK-Phytocomponent complex with BGN:

BGN-Phytocomponent complex and FAK-Phytocomponent complex were docked with FAK and BGN respectively to see the interaction energy of protein-ligand-protein complex which will be used further in the study.

(E) Molecular dynamics simulations:

The molecular dynamics simulation study was carried out to identify the changes in the structural conformation, interaction pattern and binding stability of BGN-Phytocomponent-FAK and FAK-Phytocomponent-BGN complexes. The complex was simulated with frame capture at 10ns (Production Period) intervals to analyze the molecular dynamics (MD) trajectory.

The default simulation parameters of YASARA Structure were used for the study with fixed pressure P, temperature T and number of atoms N (constant-NPT ensemble) with TIP3P (Three-site Transferrable Intermolecular Potential) [19] water model and amber 14 force field [20].

The pH of the simulation system by default is set as the physiological pH of 7.4. Furthermore the MD simulation was performed at temperature 298K , 1 bar pressure with ion concentration as a mass fraction of 0.9% NaCl ,with solvent density of 0.997 and at time steps of 1fs with the periodic boundaries wherein all the atoms present were in the mobile state. The molecular dynamics study was done to identify the stability and structural changes of the complexes in terms of time vs energy and time vs root mean squared deviation (RMSD) values [21].

RESULTS:

Using different *in-silico* tools like MVD, YASARA Structure (version 17.8.15) and Hex (version 8.0.0), the results obtained revealed a beneficial specific binding interaction between the target protein and phytocomponents which may be able to arrest metastasis by inhibiting the role of biglycan in the activation of FAK.

Molecular Docking analysis:

(A) BGN and Phytocomponents:

The ligands show binding affinity representing various types of interactions including hydrogen bond interactions, steric interactions and the responsible amino acid residues for protein-ligand interactions were His 235, Asp 237, Arg 257, Leu 258, Gly 259, Gly 261, His 262, Glu 281, His 283, Asp 285. After conformation of these key amino acid residues with all the phytocomponents, Rosmarinic acid (RA) shows the highest docking score with biglycan (Table 1). The binding poses of Biglycan and phytocomponents (Figure 3) shows that there are 5 hydrogen bond interactions with RA and the key amino acid residues were His 235, Leu 258, His 262, Glu 281 and Asp 285.

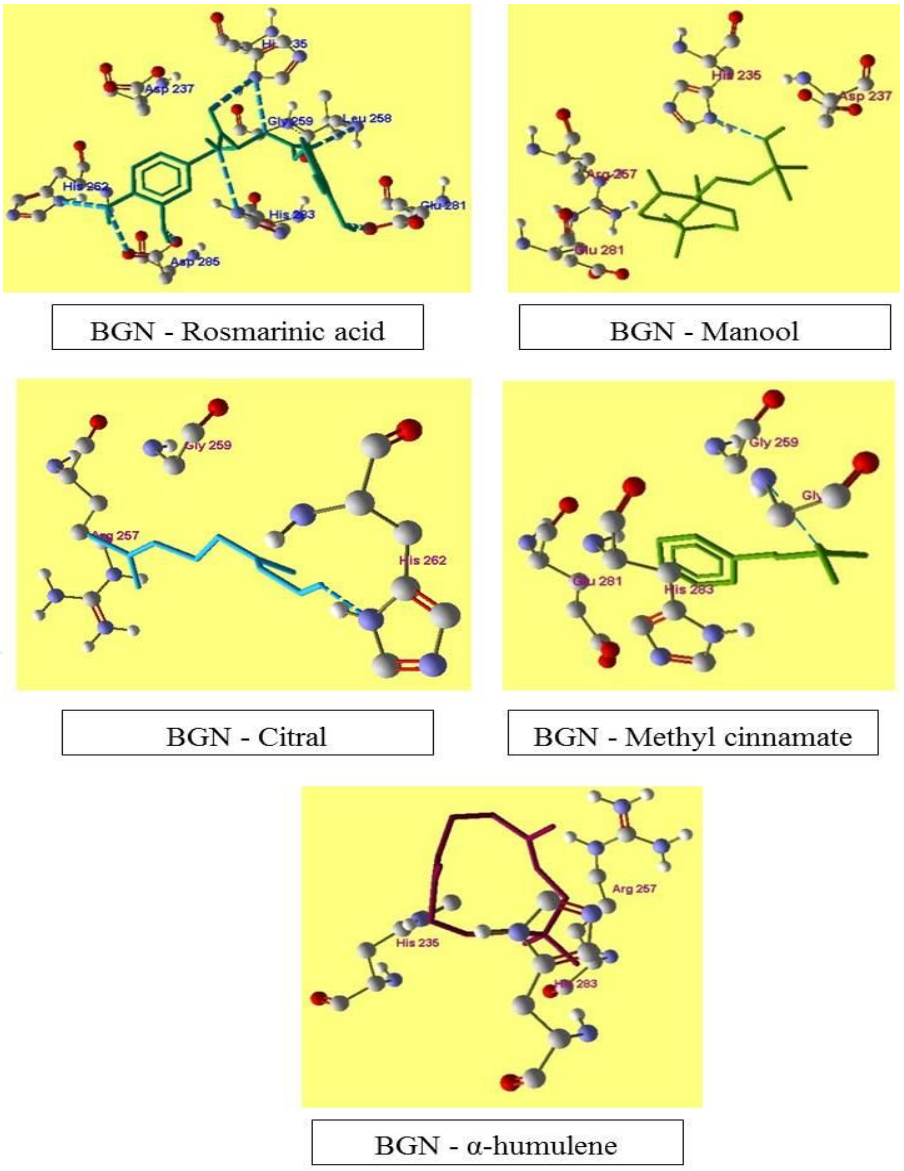


Figure 3: Binding poses of phytocomponents (Ligands) with target protein Biglycan (BGN)

Table-1: Interaction profile of Biglycan (BGN) with phytocomponents.

Protein + Ligands	Docking Score (Kcal/mol)	H Bond	Steric Interactions
BGN -Rosmarinic acid	-113.445	His 235, Leu 258, His 262, Glu 281, Asp 285	His 235, Asp 237, Leu 258, Gly 259, His 262, Glu 281,His 283, Asp 285
BGN - Manool	-88.125	His 235	His 235, Asp 237, Arg 257, Glu 281
BGN – Citral	-85.118	His 262	Arg 257, Glu 259, His 262
BGN – Methyl cinnamate	-80.631	Gly 261	Gly 259, Gly 261, Glu 281, His 283,
BGN - α-humulene	-80.254	Nil	His 235, Arg 257, His 283
BGN - β-myrecene	-78.601	Nil	Asp 237, Gly 259, Glu 281
BGN – Viridiflorol	-78.012	Nil	His 235, Arg 257, His 283
BGN - Linalool	-76.605	His 283	Leu 258, Glu 281, His 283
BGN - Carvacrol	-69.675	Leu 258, His 283	Leu 258, Glu 281, His 283
BGN - α-Thujone	-64.804	Arg 257	His 235, Arg 257, Gly 259, Glu 281

(B) FAK and Phytocomponents:

The Molecular docking study shows that FAK is effectively interacting with some of the phytocomponents and the key amino acid residues were Leu 262, Gly 263, Ser 264, Ser 265, Trp 266, Ile 268, Glu 325, Pro 326, Leu 327 and Thr 328. Rosmarinic acid (RA) shows the highest docking score in this docking study also (Table 2). The key amino acid residues like Ser 264, Ser 265, Trp 266 and Thr 328 indicated strong hydrogen bond interaction with the protein FAK (Figure 4).

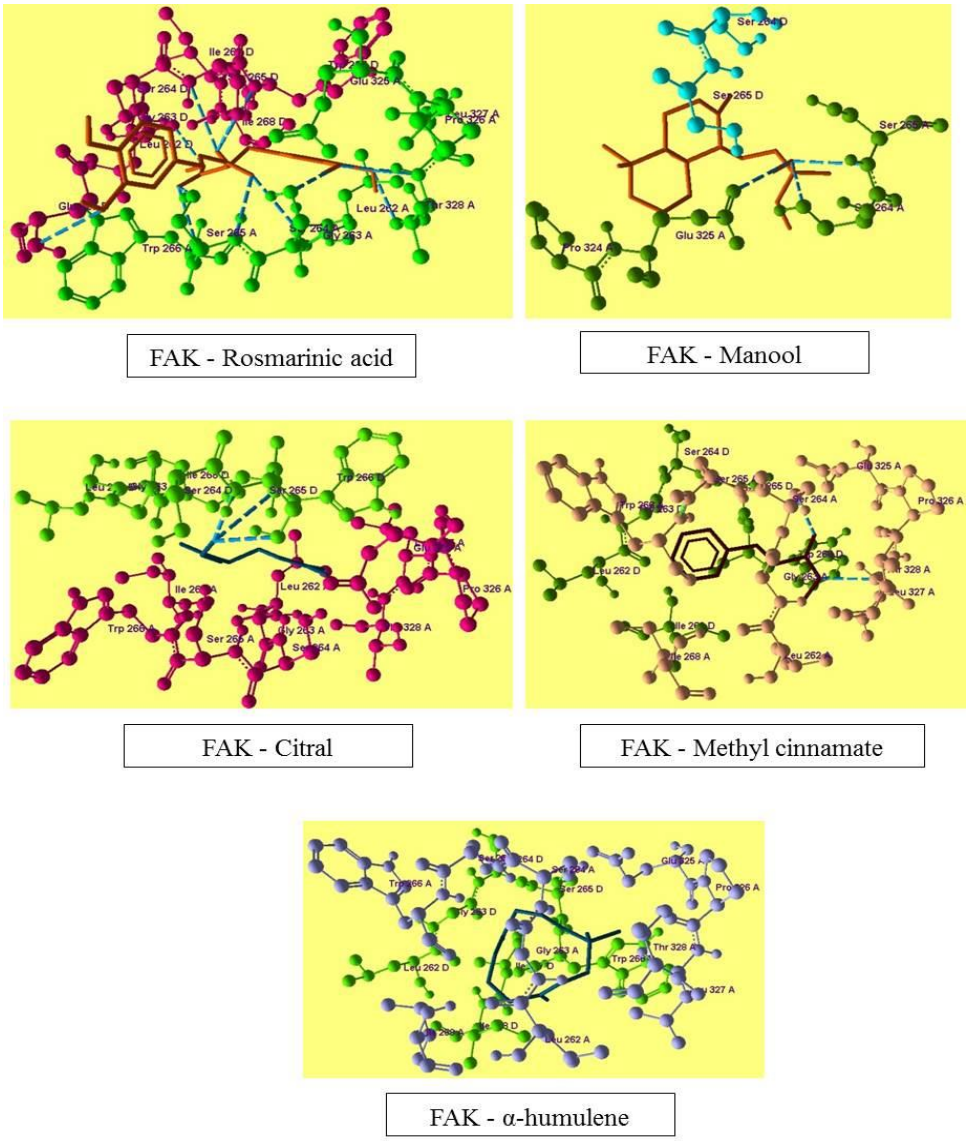


Figure 4: Binding poses of phytocomponents (Ligands) with Focal adhesion kinase protein (FAK)

Table-2: Interaction profile of Focal Adhesion Kinase (FAK) with phytocomponents

Protein – Ligands	Docking Score (Kcal/mol)	H Bond	Steric Interactions
FAK - Rosmarinic acid	-100.771	Ser 264, Ser 265, Trp 266, Thr 328	Leu 262, Gly 263, Ser 264, Ser 265, Thr 328, Leu 327, Pro 326, Trp 266
FAK - Manool	-64.236	Glu 325	Ser 264, Ser 265, Glu 325
FAK - Citral	-98.026	NIL	Gly 263, Ser 265, Leu 327
FAK - Methyl cinnamate	-101.138	Ser 264, Thr 328	Gly 263, Ser 264, Ser 265, Trp 266, Leu 327, Thr 328
FAK - α-humulene	-71.782	NIL	Leu 262, Gly 263, Ser 264, Trp 266, Glu 325, Pro 326
FAK - β-myrecene	-91.419	NIL	Gly 263, Ser 264, Trp 266, Leu 327,
FAK - Viridiflorol	-78.138	Ser 264, Ser 265, Trp 266,	Leu 262, Gly 263, Ser 264, Ser 265, Trp 266, Ile 268, , Leu 327
FAK - Linalool	-98.327	Ser 265, Trp 266	Gly 263, Ser 264, Ser 265, Trp 266, Pro 326, Leu 327
FAK - Carvacrol	-91.076	Ser 264	Ser 264, Ser 265, Leu 327
FAK - α-Thujone	-73.190	Ser 264	Gly 263, Ser 264, Ser 265, Trp 266

(C) Protein- Protein docking results:

To examine the binding interaction of biglycan and focal adhesion kinase, protein-protein docking was done using Hex (version 8.0.0) protein docking software. The result shows 500 model structures according to the interaction energy. The model which showed the highest binding interaction of-817.00 KJ/mol (Figure 5) was selected and used for further study.

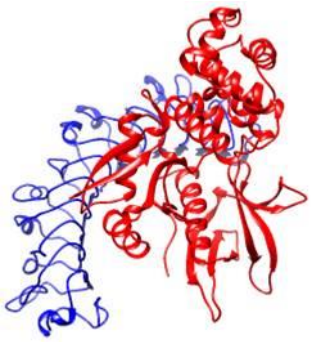


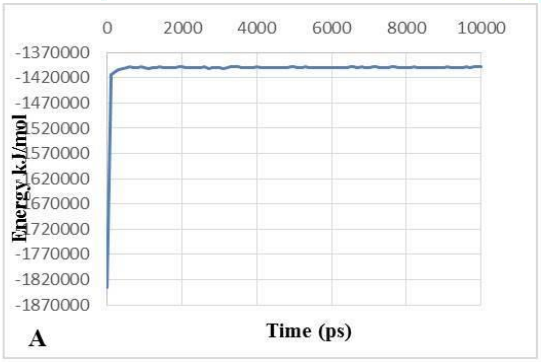
Figure 5: 3D structure of BGN-FAK complex

(D) BGN-RA complex with FAK and FAK-RA complex with BGN:

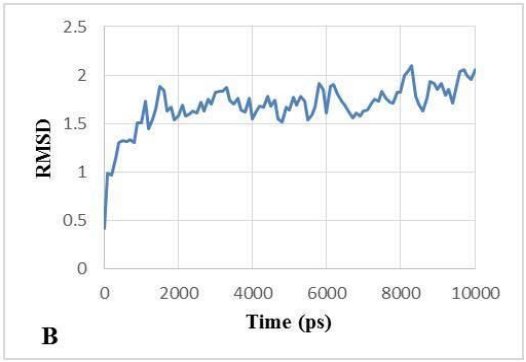
The Biglycan-Rosmarinic acid (BGN-RA) complex which was formed by the protein-ligand docking studies was then docked with FAK. The result shows that BGN-RA complex have the binding score of -763.82 KJ/ mol with the protein FAK. Similarly FAK-RA complex was docked with BGN and the interaction score noted was -815.61 KJ/ mol.

Molecular Dynamics (MD) studies:

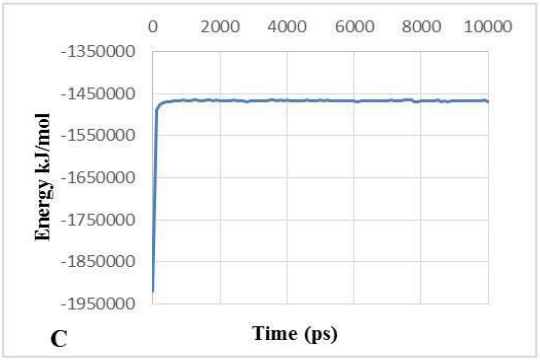
The binding poses of BGN-RA complex with FAK and FAK-RA complex with BGN were selected for the molecular dynamics study, the stability of this complex structure was analyzed with the help of molecular dynamics study using YASARA software. The study was aimed at identifying the stability of the structure which was used for this study and to observe the initial and subsequent structural changes in the complex. The results obtained indicated stability and structural changes in terms of RMSD values. Figure 6(A) and Figure 6(C) shows the MD trajectories of the complexes in Time (ps) vs Energy (kJ/ mol) having minimum -1835908.36 and maximum -1396981.43 with an average of -1403930.128 kJ/mol energy value for BGN-RA-FAK and minimum -1918312.348, maximum -1465364.668 and an average of -1472015.331 kJ/mol for FAK-RA-BGN. Time (ps) vs RMSD values can also be seen in Figure 6(B) and Figure 6(D) having major peaks at 8200ps, 8300ps, 9700ps and 10000ps for BGN-RA-FAK complex and at 7700ps, 8600ps, 9400ps, 9600ps and 10000ps for FAK-RA-BGN. Structural neighborliness relied on crystallographic protein data which was utilized to identify interfacial amino acids in the protein which play an important role in the binding interaction and could eventually aid in controlling metastasis. Molecular Dynamics (MD) simulations and interaction energy analysis confirmed the selectivity of compound as potential inhibitor based on the conformational and dynamic differences observed between the residues in the Protein active sites.



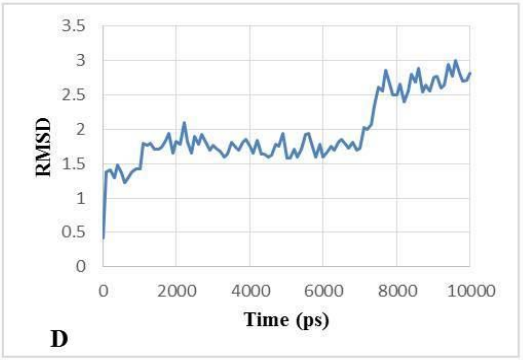
Time (ps) vs Energy (kJ/mol)



Time (ps) vs RMSD



Time (ps) vs Energy (kJ/mol)



Time (ps) vs RMSD

Figure 6: A: MD Trajectories of BGN-RA-FAK complex showing Time (ps) vs Energy (kJ/ mol)
B: MD Trajectories of BGN-RA-FAK complex showing Time (ps) vs RMSD
C: MD Trajectories of FAK-RA-BGN complex showing Time (ps) vs Energy (kJ/ mol)
D: MD Trajectories of FAK-RA-BGN complex showing Time (ps) vs RMSD

DISCUSSION:

Metastasis is one of the major causes of mortality in patients affected with lung cancer. One of the important factors of metastasis is the orientation and composition of matrix proteins. High amounts of some of these proteins favor resistance for tumor cell migration, while some other proteins and organized oriented depositions can promote tumor cell migration. Conversely, direct cell contacts between tumor cells and fibroblasts can also create a migratory-inhibitory matrix which is composed of unorganized collagens and proteoglycans [22]. Recently, Maishi and Hida [23] observed that in highly metastatic tumors, endothelial cells interact with tumor cells by secretion of a small leucine-rich repeat proteoglycan known as biglycan. Biglycan from tumor endothelial cells stimulates the tumor cells to metastasize by activating the FAK signaling pathway. FAK protein in tumors is therefore associated with increased rates of migration and invasion [24, 25]. An in-depth study by Megison *et al.* [26], suggested that the inhibition of FAK resulted in decreased cellular migration and invasion in neuroblastoma cell lines with decreased metastasis in a murine model. Zhao and Guan [27] have also explained that inhibition of FAK activity also regulates motility and cell adhesion by imparting extracellular matrix (ECM) signals from integrins to the intracellular compartment.

In the present study, selected phytocomponents from different culinary herbs manifested effective binding with Biglycan and FAK which seems to be potent in arresting the metastasis by inhibiting the role of the target protein Biglycan in the activation of FAK. These findings suggest that the selected phytocomponents could prove useful in therapeutics as blockers of key molecules that trigger metastasis. It is well known that medicinal plants are used in many countries as an alternative to synthetic drugs. Scientists are currently working towards identifying herbal extracts which could act as an active agent, to overcome world health issues [28]. These medicinal plants are rich source of phytochemicals and secondary metabolites which have been used in traditional medicine and as chemical entities for modern drugs. The medicinal value of the plant depends upon the presence of specific phytochemical components which bring about particular physiological effects in the human body. Research carried out by Ozlem *et al.,* [29] shows that the herb rosemary can inhibit the initiation and tumor progression stages in mice models. Hence in the present study, certain medicinal herbs like rosemary, sage, parsley, thyme and basil were selected, based on their potential medicinal properties.

In this study, 30 phytocomponents from rosemary, thyme, parsley, sage and basil were selected to evaluate their potential to inhibit biglycan so as to block the activation of the FAK signaling pathway and in turn, arrest metastasis in non-small cell lung cancer. Amongst these 30 phytocomponents, ten were selected according to their binding interactions with the protein for further studies with the protein complex.

According to the results obtained in the present investigation, Rosmarinic acid (RA) gave the highest docking score with biglycan and FAK hence it was selected for the molecular dynamics study. Rosmarinic acid (RA) is an abundant phenolic ester found in Rosemary (*Rosmarinus officinalis*), Thyme (*Thymus vulgaris*), Basil (*Ocimum basilicum*) and Sage (*Salvia officinalis*) which has been used in oriental medicine [30, 31]. Many studies have reported that RA possesses variety of biological effects including anti-inflammatory, anti-diabetic, and anti-cancer activities[32]. In a study by Xu *et al.* [33], it has been reported that Rosmarinic acid inhibited bone metastasis induced by breast cancer, which was substantiated in a further study [34].

Analysis of binding energy values for Rosmarinic acid yielded the highest docking score as compared to other phytocomponents. Molecular docking study of BGN-RA complex with FAK showed a binding interaction of -763.82 KJ/ mol which is less than the binding score of BGN-FAK complex which indicated that the phytocomponent rosmarinic acid effectively inhibits the interaction of BGN with FAK. The interaction energy of FAK-RA complex with BGN suggests the converse as the interaction energy is higher than that of the BGN-RA complex with FAK complex, which reveals that in the presence of this specific phytocomponent, its interaction and binding affinity is greater towards BGN. Consequently, it does not bind strongly with FAK. Molecular dynamics of these complexes showed the initial and subsequent changes in structure in terms of RMSD values. The stability of the BGN-RA with FAK complex shows that the phytocomponent interaction can inhibit the binding of BGN with FAK, hence activation of focal adhesion kinase will be impaired and thus metastasis can be controlled.

This study therefore provides evidence that Rosmarinic acid could block the binding interaction of BGN to FAK and consequently impair the activation of FAK signaling, which in turn, would effectively inhibit metastasis. This molecule would therefore prove to be of immense significance in controlling metastasis in Non- small cell lung cancer.

CONCLUSION:

Molecular dynamics simulations and interaction energy analysis confirmed the selectivity of Rosmarinic acid (RA) as potential inhibitor based on the conformational and dynamic differences observed between the residues in the Protein active sites. Investigating the binding interactions of BGN-RA-FAK complex prove significant in inhibiting the interaction of BGN to FAK which can efficiently arrest metastasis in NSCLC.

Conflict of interest statement:

There are no competing interests to declare.

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